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Research Article

Distribution and dietary habits of *Lepomis gibbosus* in Natura 2000 sites of Cyprus

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Abstract

Species introductions are widely reported as a major threat to biodiversity conservation in Natura 2000 sites. Pumpkinseed *Lepomis gibbosus* was introduced in Europe in the late 19th century and is currently listed as Invasive Alien Species of Union concern. To assess its potential impact on the native freshwater communities of Cyprus, we investigated pumpkinseed distribution and diet within 21 Natura 2000 sites. Pumpkinseed occurred in 15 out of the 22 surveyed reservoirs (68.2%), while it was recorded in only four of the 26 investigated rivers (15.4%), likely due to water availability, habitat constraints and physical barriers. Overall, this non-native fish was recorded in 12 Natura 2000 sites (57.1%). Diet analysis showed a preference for chironomid larvae, molluscs, and crustaceans, with evidence of ontogenetic dietary shifts. Fish were a minor prey, while there was no evidence of predation on reptiles or amphibians, suggesting that the impact of pumpkinseed on the conservation of native aquatic vertebrates may be negligible. Surveys also allowed to record several non-native fish species, including some predators, e.g. largemouth bass *Micropterus salmoides*, pikeperch *Sander lucioperca* and channel catfish *Ictalurus punctatus*, the diet and potential impact of which should be carefully assessed. This study highlights the importance of monitoring to mitigate the spread of non-native fish and support conservation within protected areas.

Key words: Biodiversity, diet, non-native species, protected areas, reservoirs

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Introduction

The Natura 2000 European Ecological Network was designed to support the conservation of vulnerable natural habitats and threatened species throughout the European Union (EU). The network includes Special Areas of Conservation (SACs) and Sites of Community Importance (SCIs), designated under the EU Habitats Directive (92/43/EEC), as well as Special Protection Areas (SPAs), designated under the EU Birds Directive (2009/147/EEC). The effectiveness of conservation measures aiming at halting the deterioration of biodiversity depends on several drivers of change; among them, "invasion by species", that is the successful establishment of introduced species, is one of the most frequently

reported "pressures" (negative drivers of change) by EU member states (Maes 2013; Early et al. 2016; Seebens et al. 2017; Rabitsch et al. 2020). In response, in 2014 EU institutions introduced the Regulation 1143/2014, aimed at preventing and managing the spread of invasive alien species (IAS: non-native species that spread and cause a significant change in ecosystem processes or severe economic losses; Copp et al. 2005). As protected areas aim to conserve key elements of biological diversity, the impact of IAS in Natura 2000 sites is considered more detrimental than elsewhere (Foxcroft et al. 2014).

The introduction of fish species is widely recognised as a major driver of freshwater ecosystem alteration (Moyle 1997; Cowx 1998; Dudgeon et al. 2006; Leprieur et al. 2009; Strayer 2010). While the first introductions probably date back to Roman times, a peak was recorded between the end of the 19th century and mid-20th century (Welcomme 1988). River systems facilitate the spread of non-native species often resulting in substantial ecological (Cucherousset and Olden 2011) and economic (Cuthbert et al. 2021) impacts. Efforts to combat their spread and mitigate associated damages cost billions of US dollars annually (Diagne et al. 2021).

One of the most introduced aquatic species worldwide is the North American pumpkinseed *Lepomis gibbosus* (García-Berthou et al. 2005), which was first brought to Europe in the 1880s (Maes 1898), through the so-called Perpignan–Barcelona corridor (Yavno et al. 2020). Over the past century, it has been released, accidentally or deliberately, in several water basins in the EU and is now established in all member states except for Estonia, Malta and Sweden (Copp and Fox 2007). The reasons for its introduction are manifold: for angling in France, as forage for introduced predator fishes in Iberia, as an ornamental pond fish in England, and as an aquarium species in many other countries (Elvira 2001). Sometimes it has been inadvertently transferred between water bodies along with intentionally stocked species, such as young-of-year carp, *Cyprinus carpio*, or native aquatic plants (reviewed by Zięba et al. 2020). Its high establishment success has been attributed to its tolerance to harsh environmental conditions, such as high temperatures and hypoxia (Farwell et al. 2007), as well as its high reproductive success and generalist feeding habits (Cucherousset et al. 2009).

The pumpkinseed has been reported to affect native fish (Welcomme 1988), and amphibians (Bosman 2003; Préau et al. 2017), including endangered species listed in the Habitats Directive (Rabitsch et al. 2020). There is also evidence of considerable declines in native invertebrate communities, freshwater larvae of odonates, heteroptera, diptera and trichoptera showing the greatest declines (Janssen 2000; van Kleef et al. 2008). Due to its extensive ecological impact, the pumpkinseed is classified as an "ecosystem-altering" species and ranks among the top ten introduced fish species with adverse ecological effects (Casal 2006). In 2019, it was added to the list of invasive alien species of Union concern developed under Regulation (EU) 1143/2014.

Because of widespread availability of reservoirs, which possibly promoted its establishment and dispersal (Fox et al. 2007), in southern Europe the pumpkinseed is associated with severe ecological changes and economic losses (Elvira 1998). At the southernmost edge of the EU, Cyprus is recognised as a biodiversity "hotspot" (Myers et al. 2000). The most updated (July 2024) list of Annex I and II species includes 14 plants, 19 mammals, 3 amphibians and 11

reptiles, of which 5 are (semi-)aquatic species: *Hyla savignyi*, *Pelophylax bedriagae*, *Bufotes cypriensis*, *Mauremys rivulata* and the critically endangered *Natrix natrix cypriaca* (Georghiou et al. 2024). To enhance the conservation of its species and habitats, the Republic of Cyprus has designated, until now, a total of 69 Natura 2000 areas, of which 36 are designated under the Habitats Directive, 26 under the Birds Directive and 7 under both Directives. Notwithstanding, the freshwater network of Cyprus also hosts up to 20 established non-native fish species (Elvira 2001; Hadjisterkotis 2008), of which 12 – including the pumpkinseed – have been confirmed by recent surveys (Zogaris et al. 2012; I.A.CO 2022). Additionally, two other IAS of Union concern, the pond slider *Trachemys scripta* and red swamp crayfish *Procambarus clarkii*, occur in 44 inland lentic and lotic water bodies across the island (Papatheodoulou et al. 2021). Knowledge gaps still exist regarding the potential impacts of these IAS on native freshwater communities of Cyprus. As a preliminary step to assess the consequences of introductions on conservation efforts, we investigated the distribution and diet of pumpkinseed in lentic and lotic water bodies within 21 Natura 2000 sites. Sampling was conducted in rivers using electrofishing and in reservoirs using both nets and electrofishing in shallow waters. Diet analysis aimed at assessing the potential impact of pumpkinseed, specifically on species of conservation interest.

Materials and methods

Study area

Cyprus has a Mediterranean climate, with a hot dry season from mid-April to mid-September; average annual rainfall is 480 mm/y, ranging from 450 mm in the south-western part of the island to nearly 1100 mm on the Troodos massif (1951 m a.s.l.) (Camera et al. 2014).

The study area included 21 Natura 2000 sites (30.5%) and nearby areas (Fig. 1), which were selected based on the occurrence of waterbodies and water availability. Arid sites, drained only by temporary watercourses, were excluded. Nineteen river catchments fell within the 21 selected sites, including 22 lentic waterbodies (21 reservoirs and Paralimni lake) and 67 watercourses, for a total length of 799 km. Of these, 182 km had perennial flow and 617 km temporary flow (ephemeral, harsh intermittent, intermittent). Water quality data for the period 2018–2022, as well as reservoirs characteristics, were provided by the Water Development Department (see Suppl. material 1).

Fish sampling

Three sampling campaigns were conducted from November 2022 to November 2023. Ten of the 22 lentic water bodies were sampled using both nets and electrofishing, while 12, small and shallow waterbodies, were sampled using only electrofishing. Seven gillnets with mesh width between 10 and 30 mm and two trammel nets (10 and 30 mm) were used. All nets were 2 m deep and varied in length from 18 m (10 mm) to 35 m (20 mm) and 50 m (30 mm) in length. The nets were cast in groups of three to sample different depths and habitat conditions (e.g.: rocky and muddy banks). They were set in the evening and retrieved

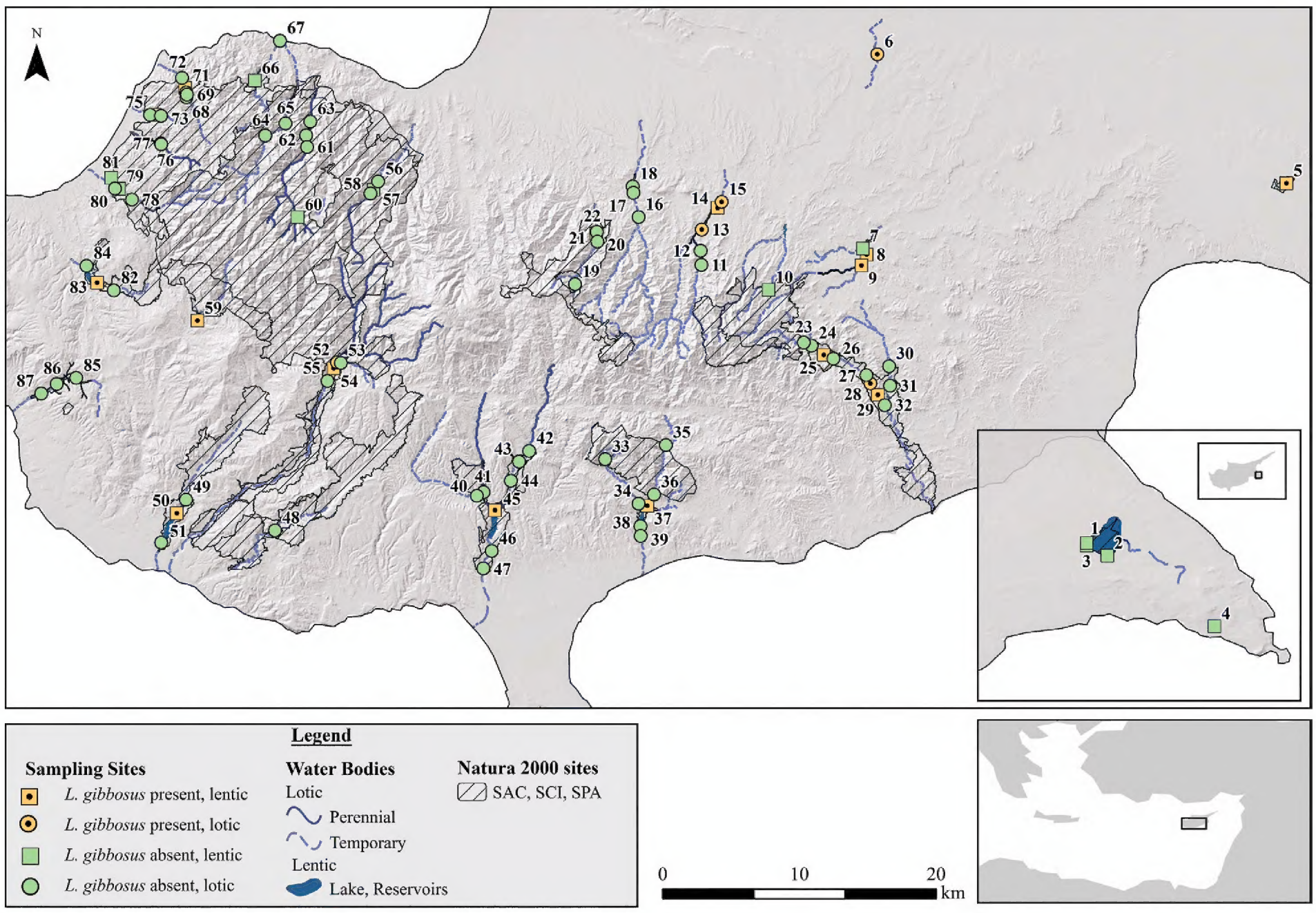

Figure 1. Distribution of pumpkinseed *Lepomis gibbosus* in the sampling sites.

early the next morning, using a small electric dinghy boat, to minimise the risk of accidental fuel pollution.

Electrofishing was carried out from the boat, using a Scubla motor-driven backpack device (ELT60 II GI_1300 Watt – 32 kW), using either direct – or pulse direct current and low – or high voltage (300–500 volts or 580–940, respectively) depending on water conductivity. Sampling was carried out along the banks, selecting, when available, potentially suitable-to-pumpkinseed stretches with aquatic vegetation and thin sediment. To uniform the sampling effort, the time-fished was set at 30 min.

Within the 21 Natura 2000 sites, 26 watercourses were selected based on previous fish sampling results (I.A.CO 2022), that is excluding those where past intensive sampling revealed no pumpkinseed occurrence. As the species was introduced into reservoirs, particular attention was given to their tributaries and outlet watercourses, with sampling by electrofishing conducted immediately upstream and downstream of the reservoirs. For all other watercourses, 1–2 suitable and accessible sites along an up to 3 km long representative stretch were sampled. For each sampling site (N = 61), a river stretch of a length at least 10 times the river's wetted width was sampled. In the absence of an approved action plan for species management, all live-caught individuals (except for those selected for diet analysis) were returned to the waterbody. Dead individuals were disposed of in agreement with competent authorities. Total weight (± 0.5 g) and fork length (± 1 mm) were recorded for each fish.

Diet analysis

At each sampling site where pumpkinseed were confirmed to occur, 10% of individuals (up to 10 individuals/site, as provided for in the permits we obtained by the competent authorities) were selected for stomach content analysis. Considering that the main aim of the analysis was to assess the impact of this IAS on aquatic vertebrates and macroinvertebrates, we selected the largest individuals, while juveniles (< 77.5 mm; Top 2012), which feed almost exclusively on zooplankton, were discarded. Selected individuals were euthanized and eviscerated in the field. Stomachs and intestines were preserved in 95% ethanol and frozen until analysis.

In the laboratory, stomachs and intestines were flushed into a Petri dish, keeping separate the relative contents, which were sorted using a stereoscopic microscope. The number of individuals of each prey item was assessed based on the number and position (left-right) of diagnostic hard parts (e.g.: mouth bones for fish, the labium and distal end of the abdomen for odonate larvae). When no diagnostic part was found, undigested remains were considered to belong to a single individual. Prey items were identified to the lowest feasible taxonomic units, using reference collections for fish and available keys for macroinvertebrates (e.g. Campaioli et al. 1994; Maasri and Thorp 2024). The contribution of each food item in terms of volume was assessed following Kruuk and Parish (1981). For each sample, the method entails estimating by eye the bulk of each item 'as ingested', *i.e.* the overall volume of each prey item. This estimate is easy and effective for small predator fish, which ingest their prey entirely.

Results were expressed as percent frequency of occurrence (F% = number of samples containing a specific food item/total number of samples x 100), percent relative frequency of occurrence (FR%: number of occurrences of a food item/total number of occurrences x 100), percent volume (V% = total estimated volume of each food item as ingested/number of samples containing that item) and percent mean volume (Vm% = total estimated volume of each food item as ingested/ total number of samples x 100), which outlines the proportional contribution of each food item to the overall diet (Kruuk and Parish 1981). The F% of main food items was plotted against their V%. In this plot xy/100 equals the mean per cent volume of each food in the overall diet (Vm%), and all points with equal xy values are connected by isopleths (Kruuk and Parish 1981). To assess differences in diet composition among fish size classes, we used permutational multivariate analysis of variance (PERMANOVA) using 999 permutations and Euclidean distances. To stabilise the variance and reduce the influence of zero values, we used Hellinger-transformed prey volumes (Vm%). We calculated Euclidean distance matrices from the transformed data and conducted the PERMANOVA. Multivariate homogeneity of variances was tested using the *betadisper* function in the vegan package (Oksanen et al. 2024), followed by ANOVA to test for significant differences. To identify which prey categories contributed most to the observed differences between size classes, post hoc comparisons were performed using Similarity Percentage analysis (SIMPER), using 999 permutations. Raw frequency data were compared using the chi-squared test.

Results

Distribution

The pumpkinseed was recorded in 15 (68.2%) of the surveyed reservoirs, for a total catch of 429 individuals (Fig. 1, Table 1). The richness of the communities ranged between 1 (Paralimni) and 9 (Gemasogeia), with pumpkinseed and largemouth bass *Micropterus salmoides* being the most widespread species in terms of frequency of occurrence (ca. 70% of lakes) and common roach *Rutilus rutilus* and channel catfish *Ictalurus punctatus* the most abundant species (27–34% of the total catch). Pumpkinseed occurrence was strongly associated with the presence of largemouth bass, the two species co-occurring in 86.6% of the surveyed reservoirs. The pumpkinseed was relatively abundant in three reservoirs – Evretou, Arminou and Germasogeia – while its occurrence was negligible in four reservoirs – Kouris, Dipotamos, Kannaviou and Agia Marina (Table 1). Electrofishing proved to be the most effective sampling method, accounting for more than 90% of the total catch and confirming the species occurrence in six out of ten reservoirs where both nets and electrofishing were applied.

In watercourses, pumpkinseed were recorded in only four rivers (15.4%) and as many sampling sites (6.6%) immediately up – or downstream a reservoir. Among permanent flow rivers, only the River Diarizos (n° 52 in Fig. 1), which feeds the Arminou reservoir, hosted pumpkinseed, although only one fish could be sampled. Six individuals were sampled in the River Maroullena (n° 13), upstream the Klirou-Malounta-Akaki reservoir and one downstream of the same waterbody, in the River Akaki (n° 15). Finally, three pumpkinseed were caught in the River Syrkatis, which feeds the Dipotamos reservoir (n° 28).

Overall, pumpkinseed were recorded in 12 Natura 2000 sites (57.1%), four SACs, two SCIs and six SPAs (see Suppl. materail 1). The mean length (± SE) of pumpkinseed was 75 ± 2 mm (N = 342; min-max = 20 – 197 mm). Most individuals (75.1%) measured less than 100 mm, while only eleven (3.2%) individuals exceeded 150 mm in length.

Diet

A total of 68 individuals were collected in the surveyed reservoirs (Table 2), ranging between 80 and 197 mm in total length (mean ± SE = 126 ± 4 mm). Most (95.6%) were caught by electrofishing and suddenly euthanized to stop the degradation of stomach contents. For most specimens, both stomach and intestine contents were flushed and analysed separately. The percentage of empty apparatuses was 22.2%. Although the content of the two apparatuses often differed within the same individual, no significant difference was recorded for the frequency of occurrence of any food item ($P > 0.16$ for all chi-squared tests). Consequently, the overall diet was assessed based on the combined analysis of 91 contents. Chironomid larvae, snails and crustaceans (crayfish and ostracods) formed the bulk of pumpkinseed diet (Vm ≈ 58%; Table 3, Fig. 2). Secondary aquatic prey consisted of odonate larvae (mainly Anisoptera) and small fish, including common roach, Eastern mosquitofish (*Gambusia holbrooki*) and pumpkinseed. Terrestrial insects, mostly ants (both swarming winged females and workers) were also preyed on. PERMANOVA showed size

Table 1. Overall composition of fish communities of the 22 waterbodies (21 reservoirs and Paralimini Lake) surveyed in 2022–2023. For those sampled by two methods, numbers are reported separately (nets – electrofishing). Site numbers (N) correspond to the numbers in Fig. 1 and Suppl. material 1.

N	Reservoir/Lake	*Lepomis gibbosus*	*Micropterus salmoides*	*Rutilus rutilus*	*Blicca bjoerkna*	*Cyprinus carpio*	*Carassius auratus*	*Alburnus alburnus*	*Ictalurus punctatus*	*Sander lucioperca*	*Perca fluviatilis*	*Oerochromis aureus*	*Gambusia holbrooki*	*Anguilla anguilla*
1–3	Paralimni	-	-	-	-	-	-	-	-	-	-	-	1	-
4	Aftelotos	-	-	-	-	-	-	-	-	-	-	-	20	-
5	Achna	36	6	-	-	5	-	-	-	-	-	16	-	-
7	Kotsiatis	-	2	-	-	-	-	-	-	-	-	-	1	-
8	Mathiatis 1	25	8	-	-	1	5	-	-	-	-	-	-	-
9	Mathiatis 2	17	2	-	-	-	-	-	-	-	-	-	-	-
14	Klirou-M.-Akaki	0–23	2–10	2	-	-	-	-	706–0	-	-	-	-	-
21	Xyliatos	26	9	-	-	-	-	-	-	-	-	-	-	-
25	Lefkara	0–18	4–0	3–0	-	-	-	-	-	-	-	-	-	-
29	Dipotamos	0–2	3–0	62–5	-	3–0	3–0	-	276–0	3–0	-	-	-	-
37	Germasogeia	14–45	12–9	50–14	19–0	-	4–0	222–8	18–0	39–0	-	1–0	-	-
45	Kouris	0–2	0–7	218–6	9–0	0–1	-	202–14	22–1	84–1	40–0	-	-	-
50	Asprokremmos	1–19	13–6	26–3	32–0	5–1	-	40–3	42–0	2–0	-	-	-	-
54	Arminou	25–81	34–13	641–1	-	-	22–0	54–5	9–0	-	-	-	-	-
57	Kafizes	-	-	-	-	1	-	-	-	-	-	-	3	-
59	Kannaviou	0–1	-	23–7	-	-	-	-	31–0	65–0	-	-	-	-
60	Tsakistra	-	-	-	-	-	5	-	-	-	-	-	-	-
67	Pyrgos	-	-	-	-	-	-	-	-	-	-	-	100	-
71	Pomos	12	4	-	-	-	-	-	-	-	-	-	-	1
74	Agia Marina	2	7	-	-	-	-	-	-	-	-	-	-	-
79	Argaka	-	-	340–12	-	-	-	-	11–4	-	-	-	0–1	0–8
83	Evretou	1–79	7–0	70–7	11–0	-	-	24–0	100–2	10–0	-	-	-	-
	Total	**41–388**	**75–85**	**1433–57**	**71–0**	**8–9**	**29–10**	**542–30**	**1215–7**	**203–1**	**40–0**	**1–16**	**0–126**	**0–9**

Table 2. Number (N) of fishes analysed for the assessment of pumpkinseed's diet. The reservoirs were sampled using either nets and electrofishing (Ef), or only the latter method (cfr. methods). Site numbers (N) correspond to the numbers in Fig. 1 and Suppl. material 1.

N	Reservoir/Lake	N	Sampling method	
			Nets + Ef	***Ef***
1–3	Paralimni			x
4	Aftelotos			x
5	Achna	4		x
7	Kotsiatis			x
8	Mathiatis recharge weir 1	3		x
9	Mathiatis recharge weir 2	2		x
14	Klirou-Malounta – Akaki	13	x	
21	Xyliatos	3		x
25	Lefkara	2	x	
29	Dipotamos	1	x	
37	Germasogeia	7	x	
45	Kouris		x	
50	Asprokremmos	4	x	
54	Arminou	15	x	
57	Kafizes			x
59	Kannaviou	1	x	
60	Tsakistra			x
66	Pyrgos			x
71	Pomos	2		x
74	Agia Marina	1		x
79	Argaka		x	
83	Evretou	10	x	
	Total	**68**	**10**	**12**

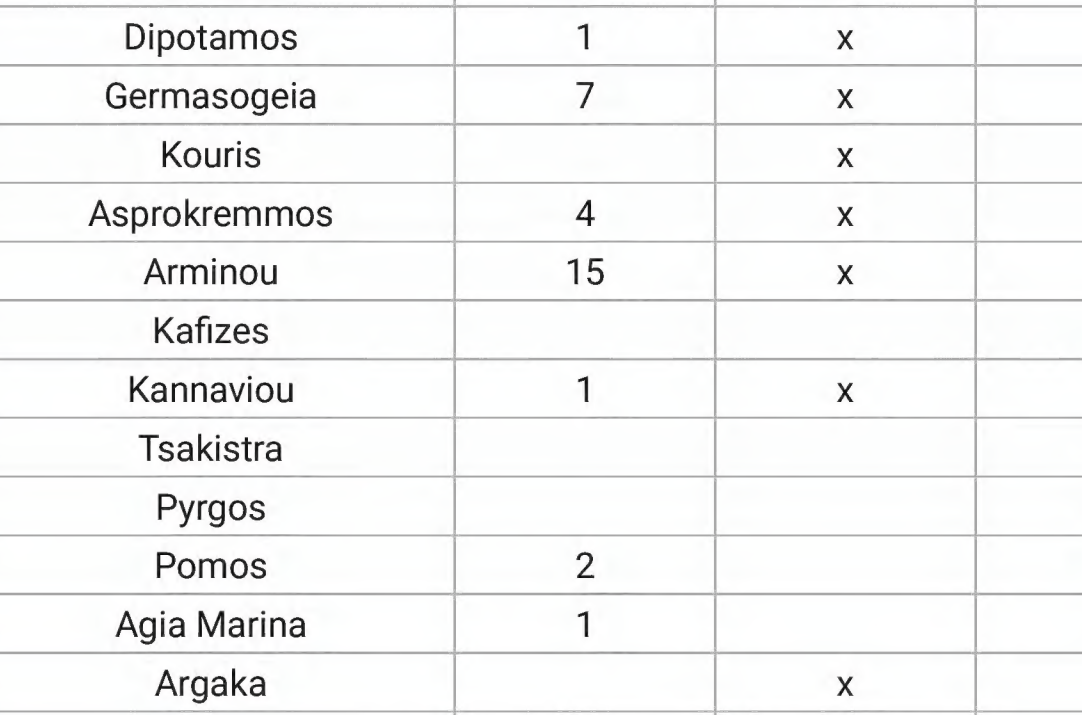

Figure 2. Overall diet of the pumpkinseed, as assessed by the analysis of stomach and intestine contents. Percent frequency of occurrence (F%) vs. the estimated percent volume (V%) of eight major food categories (diamonds: aquatic prey; squares: terrestrial prey); isopleths connect points of equal percent mean volume (Vm%) in the diet.

Table 3. Overall diet of the pumpkinseed (N = 91), as expressed in terms of both frequency of occurrence and volume (cfr. methods).

Food items	F%	FR%	V%	Vm%
Diptera larvae	56.0	29.8	67.2	37.6
Tipulidae	1.1	0.6	50.0	0.5
Chironomidae	53.8	28.7	68.7	37.0
Psychodidae	1.1	0.6	10.0	0.1
Odonata larvae	14.3	7.6	53.1	7.6
Anisoptera	13.2	7.0	47.9	6.3
Zygoptera	3.3	1.8	71.7	2.4
Baetidae larvae	2.2	1.2	25.0	0.5
Trichoptera larvae	2.2	1.2	25.0	0.5
Undet. Invertebrate Larvae	2.2	1.2	52.5	1.2
Mollusca	28.6	15.2	39.6	11.3
Lumbriculidae	1.1	0.6	100.0	1.1
Lumbricidae	1.1	0.6	100.0	1.1
Coleoptera	12.1	6.4	36.8	4.5
Undetermined	7.7	4.1	14.3	1.1
Staphylinidae	8.8	4.7	40.6	3.6
Formicidae	13.2	7.0	57.5	7.6
Odonata (adults)	3.3	1.8	30.0	1.0
Undetermined Hymenoptera	2.2	1.2	17.5	0.4
Undetermined insects	8.8	4.7	36.3	3.2
Crustacea	19.8	10.5	44.4	11.4
Ostracoda	8.8	4.7	34.4	3.0
Procambarus clarkii	11.0	5.8	76.0	8.4
Fish	8.8	4.7	70.6	6.2
Gambusia holbrooki	1.1	0.6	50.0	0.5
Lepomis gibbosus	1.1	0.6	80.0	0.9
Rutilus rutilus	1.1	0.6	30.0	0.3
Undetermined fish	5.5	2.9	81.0	4.5
Algae	5.5	2.9	64.0	3.5

class-related variations (R^2 = 0.119, F = 4.31, p = 0.001), while the betadisper test indicated no significant differences in multivariate dispersion (F = 0.65, p = 0.523). The prey items contributing most to the observed differences were Mollusca (accounting for 21% of the dissimilarity between size classes 1 and 2; p = 0.003), terrestrial invertebrates (32% of the dissimilarity between 1 and 3; p = 0.001), and Diptera (31% of the dissimilarity between 1 and 3; p = 0.003). Larger individuals preyed more frequently on terrestrial insects and less on dipteran larvae (Table 4).

Discussion

Although the occurrence of introduced species in protected areas has progressively increased since the 1980s, their impact has been long underestimated by

Table 4. Chi-squared test for the frequency of occurrence of the major food categories in the diets of three size-classes of *Lepomis gibbosus* (N = 12, 44 and 11, respectively).

Food items	Length classes (mm)			Chi^2	P
	80–100	101–150	151–200		
Aquatic invertebrates	87.0	64.9	44.0	9.69	0.008
Diptera	30.4	43.2	8.0	10.53	0.005
Odonata larvae	13.0	8.1	4.0	1.31	n.s.
Mollusca	26.1	4.1	24.0	11.9	0.003
Procambarus clarkii	0.0	1.4	0.0	0.65	n.s.
Terrestrial invertebrates	0.0	24.3	56.0	19.8	<0.0001
Coleoptera	0.0	10.8	8.0	2.73	n.s.
Formicidae	0.0	4.1	32.0	20.6	<0.0001
Fish	8.7	6.8	0.0	2.04	n.s.

relevant authorities, hindering the implementation of effective management actions (Usher 1988; Monaco and Genovesi 2014). Although Cyprus started to join the Natura 2000 network in 2004 and currently ranks among the top EU countries in terms of percentage of land area covered by this network (European Environmental Bureau 2018), our results demonstrated that efforts to fully implement the Habitats Directives have not allowed to prevent the occurrence of non-native species of Union concern in freshwater habitats. Together with largemouth bass, the pumpkinseed was widespread throughout the study area and in half the surveyed protected sites. The co-occurrence of these two North American Centrarchidae suggests that the latter may have been unintentionally introduced with largemouth bass stocks, which are highly appreciated by anglers.

The relatively small number of large-sized pumpkinseed recorded may suggest two mutually non – exclusive hypotheses: 1) the species reaches sexual maturity early during development, as reported for other low-latitude populations (Copp and Fox 2007), and 2) populations are regulated by predatory fish. Our observations provide some support to the second hypothesis as small individuals (<75 mm) tended to concentrate near the banks, wherever littoral vegetation or the rocky substrate provide some cover from predators, while large pumpkinseed occurred in the water column, where they may be more susceptible to predation by largemouth bass, pikeperch *Sander lucioperca* and catfish (Hart 1997).

While pumpkinseed populations may be regulated by inter and intra-specific predation in lentic waterbodies, their spread in tributaries appears to be hindered by both habitat requirements and presence of physical (waterfalls) or artificial barriers that affect longitudinal connectivity. Reservoirs typically overflow only during the short, rainy season, which may allow the downstream spread of non-native fish. This “drip-feeding” of river stretches from upstream waterbodies has also been reported for England, where, however, the species remains confined to lentic waters (Copp and Fox 2007). In Cyprus, the ephemeral regime of most effluent watercourses, which often dry in summer, may control pumpkinseed expansion. Nevertheless, localized, pumpkinseed populations may still exist in permanent or intermittent rivers, wherever environmental conditions are suitable. As an example, the species was recorded in the River Pediaios, which slowly flows through the centre of Nicosia, where it was likely introduced as an

ornamental fish. To prevent pumpkinseed expansion, potentially suitable areas where the species may be released voluntarily should be mapped and regularly monitored by electrofishing or eDNA analysis, a non-invasive, time – and cost-efficient method for biomonitoring (Darling and Mahon 2011).

Given the currently negligible occurrence of the species in watercourses, the diet we recorded reflects the feeding habits of the species in lentic waterbodies. As previously reported for other Mediterranean areas of introduction, chironomid larvae formed the bulk of pumpkinseed diet (Rodriguez Jiménez 1989; Godinho et al. 1997; Domínguez et al. 2002; Gkenas et al. 2019, 2021). Molluscs, which are a major food item in the species' native range (Keast 1978; Osenberg et al. 1992), and crustaceans, mainly red swamp crayfish, also contribute significantly to the diet. As reported in European introduction areas, from England (Copp et al. 2002, 2010) to Spain (Almeida et al. 2009) and Hungary (Guti et al. 1991), fish were a minor prey, while there was no evidence of predation on reptiles or amphibians. We also found no evidence that large individuals are more piscivorous than average-sized individuals, with the smallest individual containing fish remains in its stomach being 85 mm long. However, the contribution of fish to pumpkinseed diet was higher than average (mean F% = 1.0; reviewed by Collar et al. 2009). Increased piscivory may occur when macrobenthos availability is low, which could also explain the relatively high (≈ 15%) contribution of terrestrial invertebrates to the diet of large pumpkinseed. The preference for terrestrial food resources shown by large individuals (> 100 mm; see also Godinho and Ferreira 1998) further confirms the ontogenetic variation in this species' diet (Keast 1978; García-Berthou and Moreno-Amich 2000).

Net-surveys allowed to assess the overall composition of the fish communities of reservoirs, with five species – channel catfish, common roach, pikeperch, largemouth bass and pumpkinseed – occurring in nearly all surveyed waterbodies. Although, considering both its diet and mean body length, the pumpkinseed is not expected to impact the conservation of other aquatic vertebrates, the diet and potential impact of other predators, e.g. largemouth bass (Hodgson and Kitchell 1987), pikeperch (Pérez-Bote and Roso 2009) and channel catfish (Heard 1958; Hilling et al. 2016) should be carefully assessed. As an example, non-native fish predators may have contributed to the decline, and hinder the recovery, of Cyprus grass snake *N. natrix cypriaca* (Blosat 2008), whose range (ca. 70 km^2; Zotos et al. 2021) currently overlaps with three of the surveyed waterbodies: Xyliatos, Aftelotos and Paralimni. Although it is currently difficult to envision the eradication of non-native fish from Natura 2000 sites, especially considering the socio-economic implications, protected areas may help mitigate their impact on native species and ecosystems. The most cost-effective strategy is to prevent introductions through public awareness-raising campaigns (Wittenberg and Cock 2001; Pyšek et al. 2014). Since many reservoirs in protected areas are used for angling, it is crucial to avert the introduction of species into waterbodies where they do not yet occur. Natura 2000 areas can also play a key role in disseminating information on biological invasions, given the great public interest in these areas. Awareness on introductions may also be raised through the involvement of anglers in monitoring and management activities. Furthermore, to increase habitat availability for native aquatic species, a network of ponds of varying sizes could be created within protected areas (Dickman 2012; Innes et al. 2019). These

ponds should be disconnected from the hydrographic network, to prevent the spread of non-native species, and could house vulnerable native species for aiding their recovery and conservation.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

The samplings were conducted following permits granted from the Water Development Department (dated 02/01/2023) and Department of Fisheries and Marine Research (dated 11/11/2022).

Use of AI

No use of AI was reported.

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Author contributions

AP, MZ and AB contributed to research conceptualization, AP, AB, BB and MS led field activities, AB, MDO and PT carried out laboratory analyses.

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Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

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Supplementary material 1

Sampling sites, N2K areas and habitat parameters of all sampled reservoirs

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Data type: xlsx

Explanation note: The Excel file contains several sheet with the list of the 87 sampling sites, the list of the N2000 sites analyzed and the lentic waterbody characteristics.

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